REMARKS

After entry of this amendment, claims 1-15 are pending. The claims have been amended without prejudice or disclaimed and find support *inter alia* in the original claims. No new matter has been added.

Claims Rejections – 35 USC § 103

Claims 1, 4-6, 12-13 and 15 are rejected under 35 USC § 103(a) as being obvious over Köster *et al.* (hereinafter "Köster"), in view of Brown *et al.* (hereinafter "Brown").

The Examiner relies on Köster for teaching the process of claim 1 except for the step (ii) of introducing at least one universal or degenerate nucleotide at the 3'-terminus, but relied on Brown for such teaching. Specifically, the Examiner contends that Köster teaches a process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence) of n base-pairs having a (+)-strand and a complementary (-)-strand. As support, the Examiner points to Col. 35 of Köster and alleges that the region of the CFTR gene amplified by the primers of SEQ ID NOs: 13 and 14 mentioned therein corresponds to the master sequence comprising a (+)- and a (-)-strands as recited in the instant claims.

The Examiner asserts that Köster teaches the limitation of "creating a collection of single-stranded fragments of the (+)-strand of the master sequence" recited in step (i) of claim 1 because, by using one biotinylated primer and one non-biotinylated primer for PCR amplification, only the strands amplified with the biotinylated primer would be captured and retained by streptavidin-coated microtiter plate after denaturation and washing steps due to the biotin label at the 5'-end of these strands. The Examiner further points to Col. 25 of Köster for teaching contacting a target nucleic acid with specific exonuclease and DNA sequencing by Mass Spectrometry *via* exonuclease degradation. The Examiner thus alleges that Köster teaches the use of 3'-exonuclease and asserts that, when the amplified strands captured by streptavidin-coated microtiter plate are treated with 3'-exonuclease, a collection of single-stranded fragments having the same 5'-terminus with a deletion of various length in the 3'-terminus such that the collection represents (+)-strands with a length of n-1, n-2, n-3 nucleotides is generated.

The Examiner acknowledges that Köster, although teaches use of analogs such as α-thio-modified nucleotides at Col. 24, does not teach introducing at least one universal or degenerate nucleotide at the 3'-terminus as recited in step (ii) of claim 1. The Examiner, however, relies on Brown for teaching this limitation. Particularly, the Examiner points to Col. 2 of Brown for teaching universal or degenerate nucleotides. The Examiner further points to Col. 19 of Brown (Example 11) for teaching introducing at least one universal or degenerate nucleotide at the 3'-terminus of the substrate by use of a terminal deoxynucleotidyl transferase enzyme. Asserting that the substrate used therein can be the collection of strands obtained from step (i), the Examiner contends that the terminal deoxynucleotidyl transferase enzyme will act on the 3'-terminus of those strands and add the universal or degenerate nucleotides analog to this end.

As to step (iii) of claim 1, the Examiner refers back to Col. 35 of Köster and asserts that it teaches annealing of primer to (+)-strands. Specifically, the Examiner alleges that the strands amplified by the non-biotinylated primer, which were present in the wash fraction after denaturation, are to be annealed to the strands produced in step (ii), which were immobilized at 5'-end by biotin tag *via* streptavidin to microtiter plate in step (i) and contain at least one universal or degenerate base at 3'-end after step (ii). The Examiner further alleges that the biotin-immobilized strands, which are shorter than the non-biotinylated strands now annealed therewith because of the prior exonuclease digestion in step (i), would be extended to the full-length of the master sequence in the presence of extension conditions (e.g. dNTPs) using the non-biotinylated strands as templates for elongation. According to the Examiner, the newly synthesized strand hybrid would contain degenerate or universal bases in the strand that are bound to streptavidin microtiter plate.

To meet the limitation of step (iv) of claim 1, the Examiner contends that Col. 35 of Köster teaches denaturation of affinity captured double stranded DNA followed by washing, after which the non-biotinylated strands are released while the strands produced in step (iii) (biotin tagged) remain bound to the microtiter plate. Using these biotin-tagged, microtiter plate-bound strands as templates in the presence of a primer and dNTPs, the Examiner concludes that mutations at the positions of the previous universal or degenerate nucleotides compared to the master sequence are effected in the newly synthesized strands. Applicants strongly disagree with the Examiner's characterization of the cited references and traverse the rejection.

The Examiner bears the initial burden of establishing *prima facie* obviousness. *See In re Rijckaert*, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993). To support a *prima facie* conclusion of obviousness, the prior art must disclose or suggest all the limitations of the claimed invention. *See In re Lowry*, 32 F.3d 1579, 1582, 32 USPQ2d 1031, 1034 (Fed. Cir. 1994).

Moreover, it is well established that under 35 U.S.C. § 103 the Examiner must consider the reference as a whole. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). In addition, the Examiner cannot selectively pick and choose from the disclosed parameters without proper motivation as to a particular selection. The mere fact that a reference may be modified to reflect features of the claimed invention does not make the modification, and hence the claimed invention, obvious unless the prior art suggested the desirability of such modification. *In re Mills*, 916 F.2d 680, 682, 16 USPQ2d 1430 (Fed. Cir. 1990); *In re Fritch*, 23 USPQ2d 1780 (Fed. Cir. 1992). "[A] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art . . . it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements ***in the way the claimed new invention does***." *See KSR International Co. v. Teleflex Inc.*, 1741 82 USPQ2d 1385, 1396 (2007) (emphasis added). Thus, it is impermissible to simply engage in a hindsight reconstruction of the claimed invention where the reference itself provides no teaching as to why the applicant's combination would have been obvious. *In re Gorman*, 933 F.2d 982, 987, 18 USPQ2d 1885, 1888 (Fed. Cir. 1991).

It is noted initially that Köster discloses processes for detecting a particular nucleic acid sequence in a biological sample using mass spectrometer. See Köster, Abstract, and Col. 3, lines 49-51. The processes according to Köster may be used to detect the presence of mutations in a target nucleic acid sequence or to determine sequence of a relatively large target nucleic acid. Köster, Col. 23, lines 25-39 and 40-45. However, the processes taught in Köster do not concern introducing mutations into a target nucleic acid sequence. In fact, nowhere in Köster is such a process taught or suggested. Thus, contrary to the Examiner's assertion, Köster does not teach a process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence) as recited in the present claims.

Brown discloses nucleoside analogues. As known in art and stated in Col. 2 of Brown, nucleoside analogues are compounds which are capable of being incorporated in a nucleic acid chain and are capable of base-pairing with a nucleotide residue in a complementary chain or base stacking in the appropriate nucleic acid chain. Brown, Col. 2, lines 22-26. However, Brown does not teach or suggest using the disclosed nucleoside analogues to generate or introduce mutations in a target nucleic acid sequence, let alone a process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence).

It thus follows that, Köster and Brown, alone or in combination, do not teach or suggest a process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence). Because the cited references, alone or in combination, do not teach or suggest all of the limitations of the main claim, a *prima facie* case of obviousness has not been established. Accordingly the rejection should be withdrawn for this reason alone.

Moreover, as discussed above, the processes taught in Köster concern detection of mutations in a target nucleic acid or sequence determination of a relatively large target nucleic acid using mass spectrometer. The entire process, according to Köster, includes nucleic acid isolation, followed by nucleic acid amplification to obtain an appropriate quantity of nucleic acid molecules if necessary, and mass spectrometry analysis. Köster, Col. 6, lines 2-3, and Col. 14, lines 55-57. To facilitate mass spectrometry analysis, the target nucleic acid containing the nucleic acid sequence to be detected or sequenced is initially immobilized to a solid support. Köster, Col. 15, lines 23-25. Immobilization can be accomplished by using the biotin/streptavidin system. Köster, Col. 15, lines 57-61, and Col. 16, lines 7-10. Depending on the purpose, the immobilized nucleic acid may be subject to additional procedures.

For example, where the process is for detecting the presence of mutations in a target nucleic acid, the immobilized nucleic acid may be subject to further oilgo base extension reaction followed by spectrometry analysis to detect point mutations and/or small deletions/insertions in the target nucleic acid. See e.g., Köster, Example 7, Col. 34, line 59, to Col. 37, line 18. As illustrated in Example 7 of Köster, the target nucleic acid was initially amplified using primers of SEQ ID NOs: 13 and 14 to obtain an appropriate quantity of the target nucleic acid molecules for analysis. The primer of SEQ ID NO: 13 is biotinylated so the strands amplified from this primer would be immobilized on the streptavidin-coated microtiter

plate. After denaturation and washing, the strands amplified from the non-biotinylated primer would be removed from the microtiter plate, leaving the immobilized strands for further oilgo base extension reaction followed by spectrometry analysis.

On the other hand, where the process is for determining sequence of a target nucleic acid, the immobilized nucleic acid may be subject to base-specific ribonucleases to generate specifically terminated fragments from the target nucleic acid. The sequence of the target nucleic acid can then be determined by analyzing each fragment with mass spectrometer and ordering the fragments. Köster, Col. 23, lines 40-45. With this regard, modified nucleotides may be incorporated into the sequences of the target nucleic acid and the results obtained from the nucleic acid molecules containing unmodified and modified nucleotides may be compared. Köster, Col. 23, lines 57-65. Alternatively, a specific endonuclease or exonuclease may be used to generate base specifically terminated fragments. Köster, Col. 25, lines 32-35.

It is thus clear that in Example 7, Köster does not teach or suggest contacting the streptavidin-captured biotinylated strands with a specific exonuclease, such as 3'-exonuclease as suggested by the Examiner, to generate a collection of single-stranded fragments having the same 5'-terminus with a different deletion in the 3'-terminus. For at least this reason, Köster does not teach or suggest step (i) of claim 1.

Even assuming *arguendo* that one skilled in the art would have used a 3'-exonuclease to treat the streptavidin-captured biotinylated strands as suggested by the Examiner, such a treatment would have been used as part of the process for determining the sequence of the nucleic acid from which the streptavidin-captured biotinylated strands were generated as discussed above. Thus, once the specifically terminated fragments are generated after the alleged 3'-exonuclease treatment, the collection of such fragments would proceed to mass spectrometry analysis for sequence determination. One skilled in the art, following the teaching of Köster, would have no reason or motivation to treat the obtained collection of fragments with a terminal deoxynucleotidyl transferase enzyme in the presence of universal or degenerate nucleotides as taught in Brwon in order to incorporate such universal or degenerate nucleotides to the 3'-end of those fragments. Accordingly, Köster and Brown, alone or in combination, do not teach or suggest step (ii) of claim 1.

Furthermore, as discussed above, in Example 7, the strands amplified from the non-biotinylated primer are removed from the microtiter plate after the denaturation and washing steps. The immobilized strands are then subject to further oilgo base extension reaction using a detection primer followed by spectrometry analysis. It is clear from the disclosure of Example 7 in Köster that the detection primer used therein is not the strand amplified from the non-biotinylated primer. See Köster, Col. 35, lines 63-65. Nowhere in Köster or in Example 7 of Köster teaches or suggests reuse the strands amplified from the non-biotinylated primer to anneal with the immobilized strands. For at least this additional reason, Köster does not teach or suggest step (iii) of claim 1.

Additionally, as also discussed above and detailed in Example 7 of Köster, SEQ ID NO: 14 is used to amplify the target nucleic acid in order to obtain an appropriate quantity of target nucleic acid molecules for analysis. Once amplification is accomplished and the strands amplified from the biotinylated primer are immobilized on the streptavidin-coated microtiter plate, a detection primer is used for the oilgo base extension reaction after removing the strands amplified from the non-biotinylated primer. Neither the primer of SEQ ID NO: 14 nor the strand amplified from the non-biotinylated primer is used as the detection primer for the oilgo base extension reaction. Thus, it is clear that Köster does not teach or suggest step (iv) of claim 1 as characterized by the Examiner.

Accordingly, when considering Köster as a whole, the cited reference does not teach or suggest steps (i), (iii) or (iv) of claim 1. Even assuming *arguendo* that one skilled in the art would consider Köster and Brown together, the combined teaching as a whole does not teach or suggest step (ii) of claim 1. Because the cited references, alone or in combination, do not teach or suggest all of the limitations of the main claim, a *prima facie* case of obviousness has not been established for this additional reason. Accordingly, the rejection should be withdrawn.

Moreover, even assuming *arguendo* that one skilled in the art would consider Köster and Brown together, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 813, 123 USPQ 349, 352 (CCPA 1959) (the court reversed the obviousness rejection holding the "suggested combination of references would require a substantial reconstruction and redesign of

the elements shown in [the primary reference] as well as a change in the basic principle under which the [primary reference] construction was designed to operate."); *see also* MPEP § 2143.01 VI.

As discussed above, the processes taught in Köster concern detection of mutations in a target nucleic acid based on mass spectrometry, but not introducing mutations into a target nucleic acid. It is thus clear that Köster teaches a method for diagnosing DNA variations that already exist in a biological sample (e.g. patient), but not a method for creating or introducing DNA variations (i.e. mutagenesis) into a biological sample. Because the modification as suggested by the Examiner (e.g. incorporating universal or degenerate nucleotides and elongating the immobilized strands to the full length of the master sequence) would change the principle of operation of the processes taught in Köster, the teachings in Köster, even combined with Brown, are not sufficient to render the claims *prima facie* obvious. For this additional reason, the rejection should be withdrawn.

Claims 2, 7-11 and 14 are rejected as being obvious over Köster and Brown, further in view of Krokan, Short, and Zaccolo. Claim 3 is rejected as being obvious over Köster, Brown, Krokan, Short, and Zaccolo, further in view of Lutz and Cosstick & Vyle. Applicants respectfully disagree.

As discussed above, Köster and Brown, alone or in combination, do not render the main claim obvious. Since claims 2, 3, 7-11 and 14 all depend, directly or indirectly, from claim 1, they recite all the limitations of the main claim. Accordingly, Köster and Brown, alone or in combination, even further in view of Krokan, Short, Zaccolo, Lutz, and Cosstick & Vyle, would not render these claims obvious for essentially the same reasons as detailed above. For at least the above reasons, reconsideration and withdrawal of the rejections is respectfully requested.

CONCLUSION

In view of the above remarks and further in view of the above amendments, Applicants respectfully request withdrawal of the rejections and allowance of the claims. If any outstanding issues remain, the Examiner is invited to telephone the undersigned at the number given below.

Accompanying this response is a petition for a one-month extension of time with the required fee authorization. No further fees are believed due. However, if any additional fee is due, the Director is hereby authorized to charge our Deposit Account No. 03-2775, under Order No. 12810-00231-US from which the undersigned is authorized to draw.

Respectfully submitted,

By /s/ Hui-Ju Wu

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